

other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

(1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (A) and which when associated with said Fd fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said cell; and

(D) isolating said bi-specific Fab-scFv fusion protein, or

(2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein

said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (2)(A) and which when associated with said Fd fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said first and second mammalian host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said light-chain antibody fragment;

(E) combining said fragments to produce a Fab-scFv bi-specific fusion protein; and

(F) isolating said bi-specific fusion protein.

34. (Amended) A method of preparing a bi-specific Fab-scFv fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

- (1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;
- (B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (A) and which when associated with said light-chain antibody fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein said expression of Fd fragment is under the control of said regulatory regions;
- (C) growing said cell; and
- (D) isolating said bi-specific Fab-scFv fusion protein, or

- (2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;
- (B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (2)(A) and which when associated with said light-chain antibody fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second mammalian host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell, wherein expression of said Fd fragment is under the control of said regulatory regions;
- (C) growing said first and second mammalian host cells;
- (D) optionally isolating said bi-specific fusion protein fragment and said Fd fragment; and
- (E) combining said fragments to produce a bi-specific Fab-scFv fusion protein; and

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(F) isolating said bi-specific fusion protein.

37. (Twice Amended) The method of claim 33, wherein said at least one arm that specifically binds a targeted tissue is a humanized Fab fragment.

38. (Twice Amended) The method of claim 33, wherein said at least one other arm that specifically binds a targetable conjugate is a humanized Fab fragment.

39. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a peptide.

40. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a carbohydrate.

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41. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a hapten.

42. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a chelator or a metal-chelate complex.

43. (Amended) The method of claim 42, wherein said chelator is a hard base chelator for a hard acid cation.

44. (Amended) The method of claim 42, wherein said chelator is a soft base chelator for a soft acid cation.

45. (Amended) The method of claim 43, wherein said chelator is a hard base chelator that comprises carboxylate and amine groups.